1 General introduction

Root-parasitic weeds of the family Orobanchaceae Vent. threaten the production of a wide range of economically important crops in warm-temperate as well as subtropical and tropical regions. The family contains the hemiparasitic *Striga* Lour. species (witchweeds), mainly parasitising cereals, and the holoparasitics *Orobanche* L. and *Phelipanche* Pomel species (broomrapes). The latters have lost their photosynthetic ability and entirely depend on their host regarding assimilates, water and nutrient supply. *Orobanche and Phelipanche* species infest numerous dicotyledonous crops (Sauerborn, 1991; Parker & Riches, 1993).

The genera *Orobanche* and *Phelipanche* comprise more than 100 species, but only seven are considered a threat to economically important crops, namely *Phelipanche aegyptiaca* (Pers.) Pomel (= *Orobanche aegyptiaca* (Pers.), *P. ramosa* L. Pomel (= *O. ramosa* (L.), *Orobanche minor* Sm., *O. cernua* Loefl., *O. crenata* Forssk., *O. cumana* Wallr. and *O. foetida* Poir. (Musselman, 1980; Rubiales *et al.*, 2005; Parker, 2009).

1.1 Biology of *Phelipanche ramosa* (branched broomrape)

The life cycle of broomrape plants consists of two stages; a hypogeal (underground) stage and an epigeal (aboveground) stage (Lolas, 1986).

Broomrape seeds only germinate in the presence of certain germination stimulants (sesquiterpene lactones, strigolactones) released by the host-plants (Bouwmeester *et al.*, 2003). After germination, they develop a specific attachment organ (haustorium) to directly connect to the vascular system of their hosts. Via the haustorium they are able to take up mineral nutrients and assimilates (Pate & Gunning, 1972; Dörr & Kollmann, 1974). Because of their limited root system, *Orobanche* and *Phelipanche* species are also dependent on water supply by their host plant. According to Harloff and Wegmann (1993) *Phelipanche* accumulates high amounts of mannitol in order to lower its osmotic potential to a much more negative value than the one of the host. The osmoregulation process is essential to *Phelipanche* for water and nutrient uptake (Delavault *et al.*, 2002).

In the *Phelipanche ramosa*/*Nicotiana tabacum* L. system, the parasitic plant emerges above ground after an underground shoot development for approximately 45 to 55 days, flowers 4 to 5 days later, and the tiny seeds (0.3 mm in diameter, approx. 100,000 per plant) ripen after another 20 to 25 days (Lolas, 1986; Wegmann, 1999). However, this can vary according to soil types and transplanting date (Gonzalez & Rodriguez, 1981).

1.2 Distribution and host range of *Phelipanche ramosa*

Among *Orobanche* and *Phelipanche* species, *P. aegyptiaca* and *P. ramosa* have the broadest host range, including plants of the families Asteraceae Bercht. & J. Presl, Solanaceae Juss., Cannabaceae Martinov and Brassicaceae Burnett (Musselman, 1980; Qasem & Foy, 2007).

P. ramosa mainly occurs in Europe, North Africa and the Middle East (Chater & Webb, 1972; Musselman, 1986). It has also be recorded in South Africa, Cuba and the United States (Parker & Riches, 1993; Musselman & Bolin, 2008) and recently, it has been accidentally introduced to South Australia and Chile (Diaz *et al.*, 2006; Panetta & Lawes, 2007).

Branched broomrape probably spread into Europe from Central Asia with increasing cultivation of hemp between the 16th and 17th century (Koch, 1887; Demuth, 1992). Under the changing agro-climatic conditions of Western Europe, *P. ramosa* infests today at a progressing rate host crops such as *Solanum lycopersicum* L. (tomato), *Nicotiana tabacum* (tobacco), *Cannabis sativa* L. (hemp), and *Brassica napus* L. (oilseed rape) (Gibot-Leclerc *et al.*, 2003; Buschmann, 2004; Benharrat *et al.*, 2005). As hemp production had lost importance in Germany, tobacco remained the main host for branched broomrape (Gonsior *et al.*, 2004). Since the middle of the 20th century the parasitic weed did not play an important role in German agriculture any more, but it recently started to become an increasing problem in tobacco production (Figure 1.1).

Figure 1.1: Tobacco field heavily infested by *Phelipanche ramosa,* Neupotz, Germany

This is inter alia due to the meanwhile almost exclusive cultivation of Virgin tobacco varieties which can be grown in monoculture up to 10 years and therefore promote the build-up of the *Phelipanche* seed-bank in soil (Buschmann, 2004; Buschmann *et al.*, 2005). According to Billenkamp (pers. comm.), a severe infestation can cause complete yield loss in tobacco fields.
In Germany *P. ramosa* mainly occurs in the southwestern region (Billenkamp, personal communication, Figure 1.2) appearing in approximately 7-11% of the tobacco-growing area. (Schwär, Wachowski, pers. comm.).

Figure 1.2: Distribution of *Phelipanche ramosa* parasitising tobacco in Germany
X = Tobacco cultivation sites infested with *P. ramosa*

1.3 Management of *Phelipanche ramosa* in tobacco

Application of conventional control methods against *Orobanche* and *Phelipanche* species is limited due to their complex biology, *i.e.* the plant reproduces by mean of tiny and long-living seeds, their very close affiliation with the host plant, and the fact that the plants can hardly be detected before they have irreversibly damaged the crop (Joel *et al.*, 2007). Management strategies should focus on reducing the soil seed-bank and interfering with the parasite's early development stages, since most of the damage to the host is inflicted before *Phelipanche* emerges above soil.

Management strategies can be based on physical methods (weeding, soil solarisation, soil tillage, flaming, flooding), chemical methods (soil fumigation, herbicides, germination stimulants) and biological methods (resistant varieties, biological control with insects and fungi, catch crops) (Dhanapal *et al.*, 1996).

Breeding for resistance against parasitic weeds is for most host plants difficult to achieve (Rubiales, 2003) because of the complex nature and low heritability of the resistance traits. Until now neither cultivars nor species that are naturally resistant have been found in the genus *Nicotiana* L. (Slavov *et al.*, 2005), although Covarelli (2002) reported one Virgin tobacco variety to be highly resistant to broomrape.

In Germany, the most common practice to control *P. ramosa* in tobacco is by foliar herbicides (at a very low dose) that have to be applied accurately timed (when the first tubercles are developed), and the application has to be repeated at least three to four times in the growing season. Drawbacks of this management approach are possible yield losses caused by phytotoxic effects of the active ingredients on tobacco (Lolas, 1986; Covarelli, 2002) and the delayed emergence of *P. ramosa* shoots after the tobacco harvest, which can lead to a further increase of the soil seed-bank.

1.4 Biological control possibilities of *Phelipanche ramosa*

Many pathogenic microorganisms, especially fungi of the genus *Fusarium*, have been described as a potential mean to control *Orobanche* and *Phelipanche* species (Bedi & Donchev, 1991; Amsellem *et al.*, 2001b; Boari & Vurro, 2004; Nanni *et al.*, 2005; Müller-Stöver & Kroschel, 2005; Alla *et al.*, 2008) and the mycoherbicidal approach seems to be a promising management tool. None of them has been developed into a commercially available mycoherbicide yet, because either virulence was considered too low for field use (Cohen *et al.*, 2002) or scarce data is available about the efficacy under field-grown conditions.

A recently found *Fusarium oxysporum* (Schlecht.) Snyd. & Hans. isolate (FOG), obtained from diseased tubercles of an *P. ramosa* population from Germany, showed an encouraging control ability of the parasite in preliminary experiments which justified further investigations under controlled and field conditions (Figure 1.3).

Advantages of the application of a soilborne fungus are its host specificity (Amsellem *et al.*, 2001b; Boari & Vurro, 2004) and the possible destruction of all underground stages of the parasitic plant, including seeds (Sauerborn *et al.*, 1996; Thomas *et al.*, 1999a) which can contribute to lower the seed-bank every year (Joel *et al.*, 2007). However, since the influence of potential mycoherbicides is often lower and not reliable under natural conditions compared to pot experiments (Sauerborn *et al.*, 2007; Zahran *et al.*, 2008), it is important to learn as much as possible about the ecology of any potential biocontrol organism. It is also essential to find appropriate formulations which can withstand adverse environmental conditions and to enhance and stabilise the efficacy of the biocontrol agents.

Figure 1.3 *Phelipanche ramosa* shoot infested with *Fusarium oxysporum*

Müller-Stöver *et al.* (2005) observed an increased reliability of biocontrol of *O. cumana* under controlled conditions when the application of the biocontrol fungus had been combined with a second control method, the use of a chemical resistance inducer (BION®, Syngenta, Basel, Switzerland, with its active component benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester [BTH]). BTH acts as a functional analogue of salicylic acid (SA) which activates induced resistance (Systemic Acquired Resistance, SAR).